



## Review

## TADs as modular and dynamic units for gene regulation by hormones



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## ABSTRACT

During cell differentiation epigenetic processes permit the establishment of a cell type specific transcriptome by limiting the fraction of the genome that will be expressed. Based upon steady-state requirements and transcription factor expression, differentiated cells respond transiently to external cues by modulating the expression levels of subsets of genes. Increasing evidence demonstrates that the genome is organized non-randomly in a hierarchy of structures within the nuclear space, where chromosome territories are segmented into Topologically Associating Domains (TADs) and sub-domains. It remains poorly understood how this three-dimensional organization of the genome participates in the acquisition of a cell-specific program of gene expression. Furthermore, it is unknown whether this spatial framework influences the dynamic changes of gene expression that accompany alterations in the cell environment. In this review, we will discuss the impact of genome topology on the response of breast cancer cells to steroid hormones. We will cover steroid nuclear receptor mechanisms of action and discuss how topological organization of the genome, including segmentation into TADs, acts as a combinatorial platform to integrate signals whilst ultimately ensuring coordinate regulation of gene expression.

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### 1. Introduction

During the process of cell differentiation epigenetic mechanisms allow the establishment of a chromatin landscape that ensures the gene expression pattern required for specific cellular functions: maintenance of expression of house-keeping genes, silencing of genes non-related to the tissue function, activation or pausing of the cognate tissue gene network. This is accomplished by active silencing of genes that should not be expressed in a given cell type or, conversely, by maintaining in an accessible configuration genes that could be regulated in response to environmental changes [1,2]. In terminally differentiated cells the basal transcriptional network depends on the particular repertoire of specific transcription factors expressed by the cell which also determines the response to extracellular cues, for instance steroid hormones. Steroid receptors are highly expressed in reproductive tissues where they play essential roles in the signal transduction of sexual hormones. In normal mammary epithelial cells estrogens and Progesterone exert pleiotropic effects to control the proliferation/differentiation balance and paradoxically act as etiologic

determinants of breast cancer [3,4]. These hormones act through binding to their cognate receptors and can activate or repress the expression of thousands of genes in breast cancer cells. Hormone activated receptors can bind directly to the promoter of the target genes, where they orchestrate the recruitment of chromatin remodeling activities and of the general transcription machinery [5–7]. The precise mechanisms of action have been described for several responsive genes but the development of high-throughput sequencing technologies recently provided a more global vision. Indeed, genome-wide analysis of nuclear receptor binding by ChIP-Seq has shown a striking absence of direct binding of the receptors within the proximal promoter region of most responsive genes. In contrast, the major regulatory sites bound by hormone receptors behave as enhancers and exert their actions at distance from the responsive promoters [8–10]. These observations suggest an important role of the three-dimensional (3D) organization of the genome in hormonal gene regulation.

It is becoming increasingly more evident that chromosomes and genes are non-randomly positioned in the cell nucleus and the vision of a dynamic and complex organization of the nucleus is replacing the classical view of genomes as linear sequences. Therefore, a proper understanding of cell identity and behavior requires the 3D integration of nucleotide sequence information and the epigenetic states of chromatin within the nuclear space

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[11,12]. It is known that chromosomes occupy preferential territories within the cell nucleus and more recently the partitioning of the eukaryotic chromosomes in so-called Topologically Associating Domains (TAD) has been revealed [13–15]. The boundaries between TADs appear to be common between cells of different origins, suggesting that they offer a modular structural scaffold for coordination of the processing of the genetic information, notably transcription [13,16]. Changes in the levels of expression of genes appear to be correlated within TADs during cell differentiation, potentially by topologically constraining the activity of enhancers or silencers [14,17]. Those structures also play important roles in the dynamic response of differentiated cells in response to external cues [18,19]. In this review we will discuss the mechanisms of action of steroid hormone receptors in the context of the three-dimensional organization of the nuclear genome, focusing on the role of TADs in the coordination of transient gene expression changes induced by steroid hormones.

## 2. Modulation of gene expression by steroid hormones

### 2.1. Transcriptional modulation by steroid hormones

Steroid hormones, e.g. Progestins, Estrogens (notably Estradiol, E2) and Glucocorticoids, exert pleiotropic actions on a wide variety of tissues by modifying the expression of their target genes at the transcriptional level. The expression of hundreds to thousands of protein-coding genes is rapidly modified in breast cancer cells in response to Progesterone or Estradiol. Microarrays and, more recently, RNA-Seq experiments demonstrate that these hormones can repress or activate gene transcription within 1–6 h of hormone exposure [8,20,21]. Although some of those changes in transcript levels depend on post-transcriptional modifications, the development of GRO-Seq, which allows direct monitoring of transcription rates, demonstrated an unexpected wide-spread action of Estradiol in MCF-7 cells [22]. Up to 30% of transcripts expressed in these cells are affected by hormone, the majority of which with changes detected as early as 40 min after exposure. Those changes have been shown to concern protein coding transcripts as well as non-coding RNA and previously unannotated transcripts [22]. More generally, differential expression analyses suggest that these hormones can affect transiently the transcription of approximately 10–15% of expressed protein coding genes, corresponding to thousands of genes. Interestingly, many responsive genes are not randomly located throughout the linear genome but are frequently found as co-regulated clusters, with repressed and activated genes segregated within responsive domains [18,21,23,24].

### 2.2. Steroid receptors

Steroid hormones are lipophilic molecules that mainly act through binding to their cognate intra-cellular receptors, which belong to the Nuclear Receptor super-family [6,7]. Nuclear Receptors, such as the Estrogen, Progesterone and Glucocorticoid Receptors (ER, PR and GR, respectively), act as ligand inducible transcription factors which, upon activation by hormone fixation to their Ligand Binding Domain (LBD), can directly recognize specific DNA sequences (so called Hormone Responsive Element, HRE) via their DNA Binding Domain (DBD), or indirectly by interacting with other sequence-specific transcription factors [6,7]. It has been proposed that Nuclear Receptors bind preferentially in open chromatin domains that are classically seen as depleted of nucleosomes [10]. However, in contrast, we observed that the Progesterone Receptor binds preferentially to nucleosomally organized DNA sequences where it initiates profound modifications of the

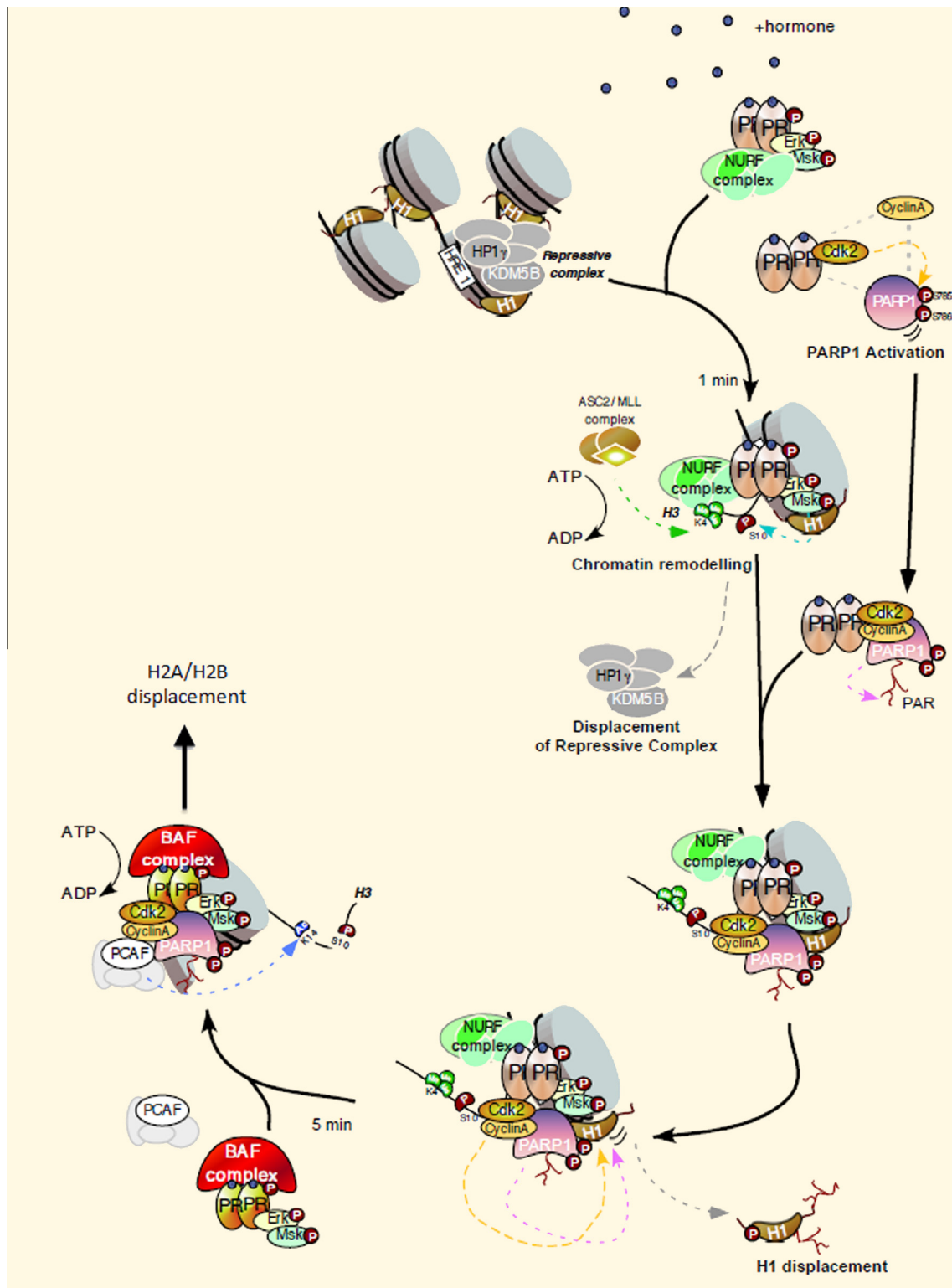
chromatin fiber [8,25]. This is made possible as hormone binding to Progesterone Receptor promotes crosstalk with kinase signaling pathways, leading to the formation of complexes containing chromatin modifying enzymes that accompany the receptor to specific regions of chromatin. Steroid hormones are known to activate various signaling cascades initiated by a small fraction of receptors anchored at the plasma membrane [26–29]. The different kinases activated through this so-called “non-genomic” pathway converge with the activated receptors to chromatin where they exert essential roles in the post-translational modification not only of the receptor itself, but also of histone tails and potentially chromatin remodeling complexes [29,30]. Indeed, once bound to their specific HRE or targeted to chromatin through protein–protein interactions, Nuclear Receptors orchestrate the recruitment of a plethora of co-regulatory proteins, including enzymes that modify the nucleosome core histone tails as well as ATP-dependent remodeling complexes involved in nucleosome sliding or eviction [31] which ultimately leads to modulation of the general transcription machinery [5].

### 2.3. Combinatorial modifications of chromatin by steroid receptors

Hormone induced remodeling of promoters occur in promoter specific and combinatorial manners. For example, the transcriptional activation of the Mouse Mammary Tumor Virus (MMTV) promoter by Progestins or Glucocorticoids requires the successive and controlled recruitment of multiple enzymatic activities [32,33]. In a first cycle, which occurs within 2 min of Progestin exposure, a NURF containing remodeling complex is recruited along with Progesterone Receptor, the ASCOM methyl transferase complex and the CDK2 protein kinase (Fig. 1). This first wave leads to the methylation of H3 on K4 and the displacement of an HP1 $\gamma$  containing repressive complex and the linker histone H1. This first step is followed by a second cycle of remodeling, which involves the acetylation of H3K14 by the histone acetyl-transferase PCAF that helps to anchor the BAF (SWI/SNF) ATP-dependent remodeling complex, which, in turn, catalyzes the displacement of H2A/H2B dimers [33,34]. The proper regulation of this Progestin-responsive model gene also involves CDK2-dependent phosphorylation and activation of PARP1, which synthesizes Poly-ADP Ribose (PAR) thus contributing to additional modification of the chromatin structure [26]. Similar profound chromatin remodeling and sequential recruitments of co-regulatory complexes have been described for other gene models in response to different steroids [32,35]. The nature and the combinations of the different complexes required, as well as their kinetics of recruitment, are highly promoter specific. Although this differential combinatorial recruitment may depend on the combinations of responsive elements within the regulatory regions of different genes, it also likely reflects differential requirements for remodeling activities, depending on the chromatin state of the promoters prior to hormone exposure [36].

### 2.4. Steroid receptors act through long-range chromatin interactions

Recent advances in the biology of steroid receptors suggest that these direct effects on promoters are seen only in a small subset of target genes. ChIP-Seq experiments unexpectedly showed that Nuclear Receptor binding to chromatin occur also within gene bodies and in inter-genic regions. In general, Nuclear Receptors bind to many sites located far away from the genes they regulate. This is notably the case for the Estrogen, Progesterone and Glucocorticoid Receptors, for which only around 10% of sites are located at less than 5 kb of promoters. The number of binding sites is an order of magnitude higher than the number of responsive genes. However, a large proportion of the genes regulated by



**Fig. 1.** Progestins induce rapid and important changes in chromatin at and the level of the promoter of responsive genes. These events involve the combinatorial and successive action of several remodeling complexes which leads to the displacement of repressive complexes, modification of the chromatin structure by eviction of linker histones and reorganization of nucleosomes.

hormones do not exhibit receptor-binding sites within their proximal promoters [8–10]. Although it might reflect secondary or indirect regulation of those genes, GRO-Seq experiments performed in MCF-7 in response to Estradiol showed that around 50% of the

transcripts modified during the first 40 min of treatment lacked proximal ER binding sites [22]. In many cases the transcriptional regulation of steroid target genes seems therefore to require the action of regulatory sequences located further away from the

promoters [8,9,22]. Supporting this view, ChIA-PET experiments performed using antibodies directed against the Estrogen Receptor in MCF-7 cells revealed important networks of interactions between Estrogen Receptor bound sites after 30 min of treatment with Estradiol [37]. Thus it appears that a significant fraction of these distal binding sites engaged interactions with promoters, suggesting they act as enhancers.

Traditionally, enhancers are viewed as sites where the recruitment of transcription factors can promote transcription of target genes located further away, either upstream or downstream. Most of them have been shown to act through physical interactions with the target promoters by chromatin looping [38]. Although individual enhancers are well known regulators of specific genes, the emergence of High Throughput methodologies, either ChIP-Seq or Chromosome Conformation Capture (3C)-derived methods [39], uncover a much more complex view. It appears that enhancers not only act on the closest gene promoter, but are also engaged in complex interaction networks with several promoters and other enhancers [17,19]. These observations strongly suggest that long-range regulation is an essential principle, shaping the transcription landscape as well as its transient modifications in response to changes in the cell environment. Recent analyses suggest that the activity of enhancers and regulatory elements is mainly restrained within sub-megabase scale domains corresponding to Topologically Associating Domains (TADs) [13–15,40,41].

### 3. TADs as modular units for gene expression

In mammals, TADs have been identified by 5C and Hi-C experiments as 1 Mb-sized domains of high local frequency of interactions and separated from each other by sharp boundaries [13,14]. TAD borders are characterized by enriched loading of structural proteins, like CCCTC-binding factor (CTCF) and cohesins, as well as by a high density of actively and ubiquitously expressed genes [13,40,42]. The location of TADs borders appear to be similar between cell types since more than 70% of them are shared between cells of different origins [13]. TADs appear to functionally organize the genome. For example, they have been shown to correspond to individual units of replication, which show cell specific kinetics of replication timing [43]. In addition, some TADs overlap with LADs (Lamina Associated Domains), domains of the genome associated with the nuclear lamina that contain transcriptionally inactive genes. TADs have also been shown to overlap with large chromatin blocks enriched in repressive histone marks as H3K9me3 or H3K27me3. In contrast, other TADs are characterized by high transcriptional activity with most genes covered by histone marks corresponding to an active state (e.g. H3K36me2/3) [13,14,16]. Thus TADs tend to exhibit homogeneous chromatin states, either active or repressive, which determine their association to spatially segregated chromatin compartments [16,44]. TAD boundaries therefore limit the spreading of chromatin modifications and separate domains with divergent degrees of permissiveness for transcription (see Fig. 2A). However, the epigenetic signature of TADs does not seem to be determining factor for their formation, since boundaries are maintained between TADs that share similar chromatin states and, conversely, absence of H3K9me3 domains in G9a deficient mice does not lead to the disappearance of the boundaries [14]. This suggests that TAD boundaries are important structural regions that do not depend on the internal chromatin state but that are used to isolate genomic elements in a combinatorial way depending on the cell type. Indeed, the epigenetic signature of TADs differs between cell types,

suggesting a role of these structures in patterning the cell specific chromatin landscape (Fig. 2A; [16]).

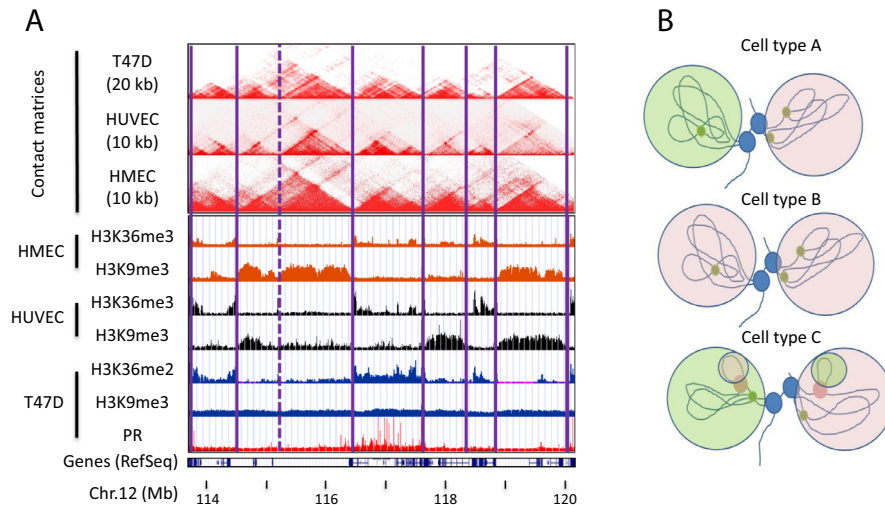
The relatively homogeneous chromatin state of a TAD likely reflects the local enrichment of distinct chromatin remodeling and histones modifying activities. Further cell specific mechanisms will therefore establish the final pattern of gene expression within the global environment of each TAD. This is likely achieved by the formation of specific loops that will either exclude genes or group of genes from the general influence and/or attribute specific regulatory sequences to a given gene or group of genes. These cell-specific loops may explain the formation of different sub-TAD structures in different cell types (Fig. 2A and B). Indeed, high resolution 3C-derived experiments have demonstrated that TADs can be further internally compartmentalized in sub-domains and loops, which boundaries might depend on different insulators and regulatory proteins combinations [41,45]. Interestingly, these secondary boundaries are more dynamic and differ between cell types. However, although they generate further compartmentalization and specificity, it appears that these sub-domains enter in contact more frequently with other sub-domains within their own TAD, indicating that they do not form totally independent structures (Fig. 2A and B). It is therefore tempting to propose that TADs represent cell invariant structural scaffolds that facilitate the formation of cell-specific functional loops meanwhile they also limit the possible functional interactions that could be engaged.

Intuitively, the property of TADs to be isolated from each other makes plausible that the activity of enhancers could be restricted to genes located within the same TAD. Indeed, most enhancers/promoters loops observed genome-wide occur between elements located within a single TAD [19,46,47] and as a consequence the genes within a TAD frequently show correlation in their activity [14,18,19]. However, the segmentation in TADs also allows a precise and tunable action of intra-tads enhancers on genes located close to the TAD boundaries, as it has been described for the HOXD cluster [48].

TADs thus offer a modular scaffold to shape epigenetic domains. By allowing changes in chromatin in a modular way during differentiation, the segmentation of the genome in TADs might be essential to the proper patterning of gene expression [13,16]. Such a view is interesting as the preferential location of house-keeping genes close to the boundaries between TADs, a more permissive area for transcription and common to various cell types, will favor their ubiquitous expression [16,40]. Conversely, tissue-specific genes, located further away from the TAD borders, will be more prone to be influenced by the global chromatin state of the TAD. The existence of regions with distinct permissivity for transcription may contribute to and provide a topological scaffold to the frequent coordinated expression observed throughout the genome [49,50] as well as to the large scale changes in chromatin occurring during differentiation [51,52] or during malignant transformation [53,54].

The high frequency of contacts within TADs is probably reflecting facilitated collisions as a consequence of both more focal specific interactions between regulatory elements within domains and insulation from neighboring region. Although this topological organization probably does not directly determine the transcription level of the individual resident genes, it could globally influence their expression by its relative permissiveness and accessibility to regulatory proteins. This implies that different regulatory mechanisms would be required to dynamically modify gene expression within different type of TADs in response to transient changes of the cell environment.





**Fig. 2.** (A) Example of TADs as defined in T47D breast cancer cells (top). Purple lines indicate the TADs borders (defined in these cells, [18]), which are similarly positioned in other cell types (HUVEC and HMEC (middle and bottom respectively; [45]; <http://www.aidenlab.org/juicebox/>)). The borders separate chromatin domains with distinct chromatin signatures and transcriptional activities as demonstrated by the relative enrichment in H3K9me3 and H3K36me2/3 (UCSC genome Browser view of ENCODE Consortium data for H3K36me3 and H3K9me3 in HMEC and HUVEC (Bernstein – Broad Institute) as well as H3K36me2 and H3K9me3 in T47D cells; [18]). The epigenetic signature and the relative enrichment in epigenetic marks of the different TAD differ between cell types in a combinatorial manner. Within the TADs further loops (dashed lines) define subdomains, which are formed in a cell specific manner and harbor specific activity. Those sub-domains remain separated from the adjacent TADs and more prone to enter in contact with the rest of the cell invariant TAD. This organization once established in a given cell type constrains the accessibility and activity of inducible transcription factors, which could bind at high density in some specific TADs, as for example the Progesterone Receptor (PR) in T47D cells. (B) Schematic representation of the combinatorial organization of TADs in different cell types. Blue circles represent cell type invariant TADs borders. Depending on the cell type, TADs belong to active (green) or inactive compartment (red). Further internal cell specific loops are formed (green and red circles) that could lead to partial insulation of subdomains.

#### 4. Coordinated changes in gene expression upon exposure to steroids

##### 4.1. Clustering of steroid responsive genes and large-scale response to hormones

As for other transcription factors, the loops involving Steroid Receptor bound enhancers are mostly restricted within TADs [8,18,37,55]. In addition, Steroid receptors binding sites are frequently found clustered or densely concentrated over large domains of the genome corresponding to TADs ([9,18]; Fig. 2). Steroid responsive genes are frequently found clustered in discrete regions of the genome where the opposite response is absent leading to segregation of activated and repressed genes in distinct TADs [18,21,23,24]. These responsive clusters correlate with high density of receptors binding sites within TADs [18]. Probably not all sites are functional, however, they can also correspond to super-enhancers that act coordinately on the expression of the surrounding genes [56]. Although some of these binding events might only reflect facilitated accessibility of dedicated chromatin hubs [10,57], it is probable they cooperatively act on the regulation of the neighboring genes, as shown by the complex interactions between ER binding sites from ChiA-PET [18,37]. In addition to the changes in chromatin described above, transcription of enhancer RNA (eRNA) is also induced within the underlying bound regions [58]. These epigenetic effects could therefore spread over larger domains and might contribute to the coordinated regulation of the neighboring genes [24,51,59]. Such large-scale coordination of response is likely not restricted to steroid but may occur in response to other signals [49].

The mechanisms by which steroid hormones lead to gene repression are less understood and may involve distinct binding kinetics of receptors as compared to activated genes [22]. However, hormone-repressed genes are also found clustered, suggesting coordinated events occur within those regions. One could hypothesize the existence of silencers that will act by recruiting repressive co-regulatory proteins. Conversely, the repression of

these genes could also be the consequence of a destabilization of enhancers that were active prior to hormone exposure. This is suggested by the observation that TADs enriched in negatively regulated genes show a decrease in long-range interactions after hormone exposure, whereas these interactions increase in TADs activated by hormones [18].

##### 4.2. Stable and dynamic contacts within TADs

Chromatin loops between enhancers and promoters are probably highly dynamic and an active enhancer might stochastically contact with all genes located in its field of action within its TAD. In addition, the chromatin modifications that fire at enhancers following the recruitment of remodeling complexes can also spread over large distances, leading to large-scale modifications. Obviously these events will contribute to coordinated changes in gene expression and in the frequent transcription ripples observed in response to various signals [49]. As mentioned, TADs and sub-domains adopt preferential signatures depending on the cell type. Once established, this organization will constrain the pattern of gene expression and the response to external cues that may require structural changes. Conversely, the changes in chromatin that accompany modifications of transcription rate will affect the possible changes in 3D organization of TADs. Recent high resolution Hi-C experiments in human primary fibroblast showed that regulatory loops between promoters and enhancers are found within TADs prior to the binding of transcription factors activated upon Tumor Necrosis Factor (TNF)- $\alpha$  exposure, suggesting that pre-organized environment will favor the response to external cues [19]. Indeed, the transcriptional response did not seem to involve stimulus induced reorganization of the loci since the interactions changes were limited after treatment with TNF [19]. Facilitated organization of domains was also observed in analysis of the response to Glucocorticoid in mouse cells by 4C. Glucocorticoid sensitive genes were organized in chromatin hubs mainly stable before and after treatment [57]. Those hubs probably reflect the TADs organization, as their boundaries have been shown

to be stable upon hormone exposure [18,19]. However, the number and the strength of the interactions between genes were increased upon treatment with the hormone in this case, suggesting a dynamic consolidation of pre-settled domains [57]. In contrast, 3C experiments performed on other hormone responsive genes suggested that the receptors are actively involved in generating functional looping between distal regulatory sites and promoters upon exposure to the hormone [8,37,60,61]. In addition, TADs responding to Progesterins also showed a dynamic redistribution of internal interactions in correlation with the transcriptional response of TADs [18].

Thus, although the existence of a preset organization seems to be a common theme for gene response to external cues, the extent of dynamic changes might depend on the type of stimulus and the domains considered. Several non-mutually exclusive mechanisms probably allow the regulatory interactions to occur (Fig. 3). It is probable that, upon binding, the receptors might stabilize loops that are favored by a more general topology of the domains: some

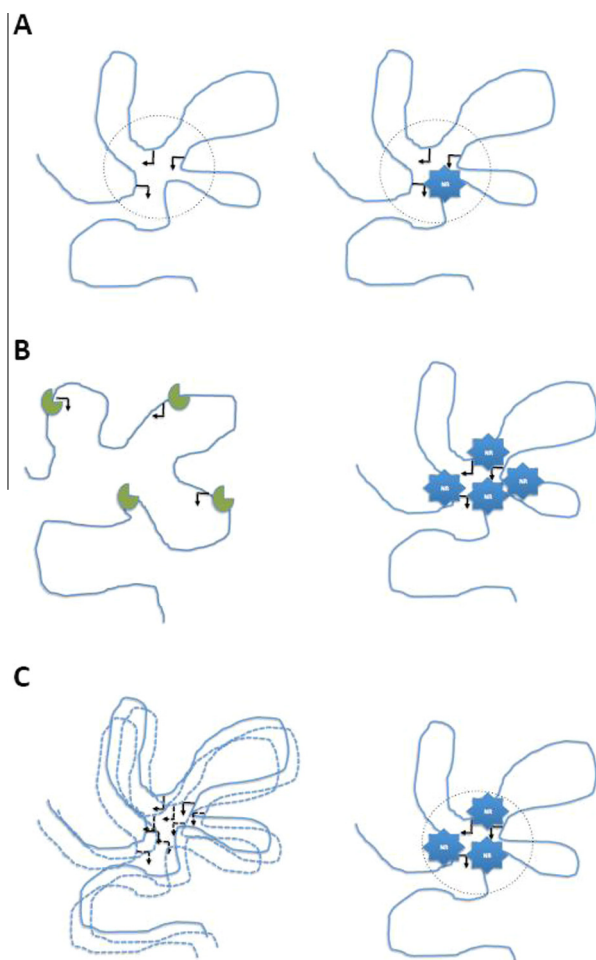
enhancer-promoter loops might be pre-settled prior to signal exposure, either engaged by non-liganded receptors or additional pioneer factors. Binding of the activated nuclear receptors within this pre-organized conformation will lead to the recruitment of additional co-regulatory factors leading to the stabilization of the structure. Conversely, the binding of the receptors could precede the formation of promoter-enhancer loops which are formed as a consequence of the changes in chromatin fiber initiated at the sites of binding (Fig. 3). Most of the effect of those distal enhancers probably require looping to the promoter and the looping itself depends on the activity of the receptor on chromatin (for example by modifying the flexibility of the chromatin), but other factors are likely required for stabilizing those interactions. Known bridging factors, CTCF or cohesins, may also be involved in the stabilization of the loops, as altered expression of these factors leads to dysregulation of genes in response to Estradiol [61–63].

## 5. Future directions

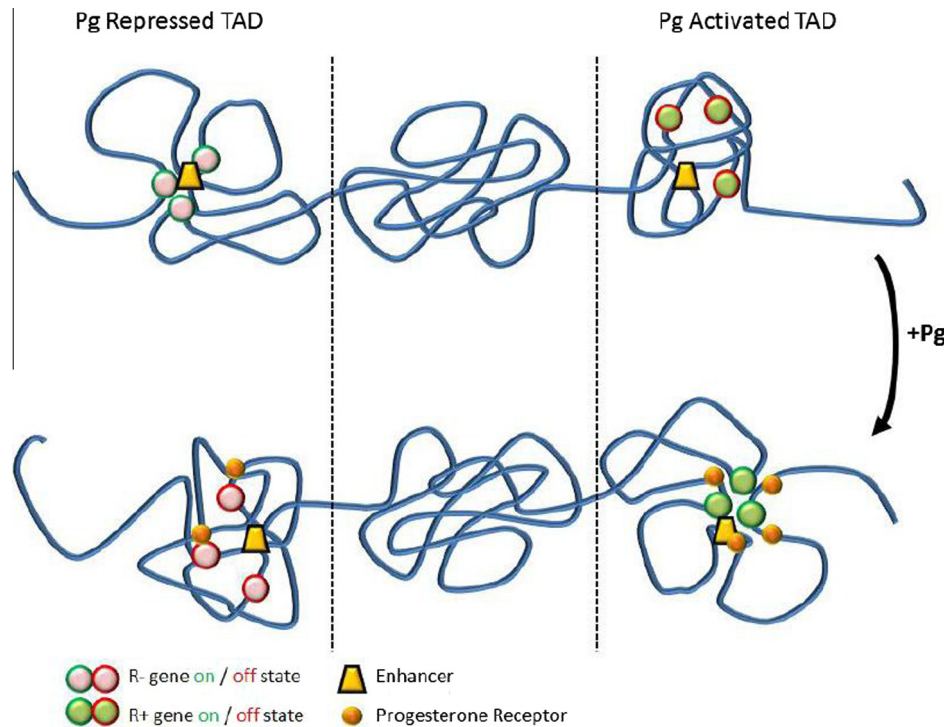
Depending on the chromatin state and the repertoire of transcription factors in different cell types, TADs could respond differently to various signals and could therefore be considered as transcriptional unit of response to external cues. This is supported by the fact that genes within a given TAD are coordinately activated or repressed in response to distinct steroid hormones (Fig. 4) [18].

One could hypothesize that the nature of the structural changes that accompany the modifications in transcription will depend on the general permissivity of the nuclear environment of TADs, including their localization within the nuclear space. For instance, due to the heterogeneous and frequently non-random distribution of regulatory proteins in the nucleus, it is probable that the mechanisms necessary for maintaining genes or group of genes in a repressed state will differ between TADs located within the nuclear interior and TADs located close to the nuclear periphery, in particular those attached to the nuclear matrix and corresponding to LADs. In such view, the link between chromatin state and nuclear positioning remains to be better understood. It will be important to determine the role of the nuclear matrix, not only nuclear lamina, but also the internal fibrillar components of the “nucleoskeleton”, in maintaining the boundaries between chromosome domains and in organizing their interactions within the nuclear space.

Although the topological organization of the genome in TADs and sub-domains appears to play a role in transcriptional response, those models are constructed from correlations. Future comprehensive understanding on the relation between expression and structure will require functional testing by genome engineering. In particular, CRISPR/Cas9 methodology [64] will permit to characterize the consequences of deletion or generation of topological borders. In addition, these methods will serve to modify the natural location of genes and determine the consequences on their expression and response to external cues. In particular it will be important to analyze the behavior of genes normally non-responding to steroids when introduced within a domain on which hormones have a more general effect. Such experiments will permit to understand the mode of actions of enhancers, regulatory elements and insulators. Finally, it is important to keep in mind that structural data obtained by Hi-C are averages of structures occurring within the cell population (see Junier et al., this issue for further reading on those aspects). It will be essential to complete this view with single cell analysis to determine whether the apparent changes induced by hormones are reflecting the transition from one structure to another in single cells or rather changes of the proportions of already established structures within the cell population. Answering this question will require



**Fig. 3.** Different models of dynamic reorganization of promoter/enhancer loops upon steroid exposure within TADs. (A) In absence of hormone enhancer (blue circle) and promoters are already found organized in networks of interactions. Binding of the receptors (blue star) upon hormone exposure favor the recruitment of coactivators without modifying the already established organization. (B) Receptor binding to promoter(s) (green elements) and enhancer sites (blue circle) upon hormone exposure is necessary to generate regulatory loops between the regulatory elements. (C) The TAD is organized in a stochastic way where interactions between enhancers and promoters are favored but remain highly dynamic. Binding of the receptors within the region stabilized these interactions either by tethering specific elements or modifying the chromatin fiber flexibility.



**Fig. 4.** Model of coordinated changes in gene expression upon progesterone. Within a Progesterone-repressed TAD, prior to hormone treatment, long-range interactions with an active enhancer maintain the transcription of the genes. Upon hormone exposure Progesterone Receptor-mediated modifications of chromatin or competition between the Progesterone Receptor and other transcription factors destabilize those interactions, leading to the repression of the expression of the genes (R-: Progesterone repressed genes). Conversely, within a Progesterone-activated TAD, Progesterone Receptor binding favors the establishment or stabilization of long-range interactions between enhancer and genes (R+: Progesterone activated genes).

complementing Hi-C experiments with high resolution microscopy or 3D-FISH analysis.

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